Clinical and Experimental Rheumatology 2004; 22: 617-620.

Increased dermal elastic fibers in the tight skin mouse

S. Chatterjee, M.E. Mark, P.H. Wooley, W.D. Lawrence, M.D. Mayes

Division of Rheumatology, Department of Internal Medicine; Department of Orthopedic Surgery; and Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan, USA

Soumya Chatterjee, MD, MS, Department of Rheumatic and Immunologic Diseases, The Cleveland Clinic Foundation, Cleveland, OH; Mina E. Mark, MD, Philadelphia, PA; Paul H. Wooley, PhD, Department of Orthopedic Surgery, Wayne State University, Detroit, MI; W. Dwayne Lawrence, MD, Department of Pathology and Laboratory Medicine, Brown Medical School, Women & Infants'Hospital of Rhode Island, Providence, RI; Maureen D. Mayes, MD, MPH, Division of Rheumatology, University of Texas Health Science Center, Houston, TX, USA.

Please address correspondence and reprint requests to: Soumya Chatterjee, MD, MS, Department of Rheumatic and Immunologic Diseases, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Desk A-50, Cleveland, Ohio 44195, USA.
E-mail address: soumyac@umich.edu

Note: Interested individuals may contact the author directly to obtain a copy of Figure 1 in colour (digital format).

Received on December 3, 2003; accepted in revised form on April 26, 2004.

Key words: Scleroderma, animal models, mice, collagen, elastin.

ABSTRACT

Objective. *The tight skin (Tsk-1) mouse has been proposed as a model for systemic sclerosis on the basis of increased accumulation of collagen and glycosaminoglycans in the skin, and by the presence of serum autoantibodies. The genetic basis of the mutation has been identified as a genomic duplication within the fibrillin-1 (Fbn-1) gene that results in a larger than normal Fbn-1 transcript, but the mechanism that leads to dermal fibrosis is unclear. Fibrillin molecules associate into a polymer that is coated with elastin molecules to form elastic fibers. To further evaluate the Tsk-1 mouse model of scleroderma, we have studied elastic fibers in the skin of these mice.*

Methods. *Skin sections obtained from C57BL/6-TSK+ (Tsk-1) and C57BL6-pa/+ (control) mice were stained with Masson's trichrome for evaluation of collagen and Gomori's aldehyde fuchsin stain for elastic tissue. Computer assisted image analysis was performed to quantify differences in histologic sections.*

Results. *Tsk-1 mice had a highly significant increase in the percentage of elastic fibers (19.6%) in the dermis compared to control mice (7.9%) [$p < 0.001$]. This correlates with the findings in the skin of systemic sclerosis patients where increased elastic fibers have been observed. In addition, an increased level of dermal collagen staining was also observed in the Tsk-1 dermis (82.9%) compared with the level in normal sections (73.7%) [$p<0.01$].*

Conclusion. *These data support the use of the Tsk-1 mouse as a model for the connective tissue abnormalities of human scleroderma.*

Introduction

Animal models are of great value in studying the pathogenesis of human diseases, and can be useful in evaluating potential therapeutic modalities for these diseases. The tight skin mouse-1 (Tsk-1) is the most extensively studied model of human scleroderma (SSc) from the genetic, histologic, immunologic and biochemical viewpoint (1). The collagen content of the skin from the Tsk-1 mice is significantly increased when compared to normal (+/+) littermates (2). Fibrillin-1 (fbn-1) is a ubiquitous protein present in the extracellular matrix of various organs and provides the core for elastic fibers. The genetic defect of the Tsk-1 mouse consists of a 30 - 40 Kb genomic duplication within the fbn-1 gene that results in a larger than normal in-frame fbn-1 transcript (3). In SSc, the volume of dermal elastic fibers is increased per unit area relative to controls, though the individual elastic fibers appear smaller, more numerous and polymorphous (4). We know that collagen deposition is increased in the dermis in human scleroderma (4) and in the Tsk-1 mouse model (2, 5). We are also aware that the dermal elastin content is increased in human scleroderma (4). However, though data are available about the paucity of elastin in the lungs of the Tsk-1 mouse [accounting for the characteristic emphysematous changes (6)], no data exist to characterize the dermal elastin content in the Tsk-1 mouse. Hence, it is not known whether the dermal elastin content in the Tsk-1 mouse model is increased (similar to that in human scleroderma), is reduced (parallel to the changes in the lungs in this mouse model), or remains unchanged. We studied dermal elastic fibers of Tsk-1 versus control mice to determine if abnormalities in these fibers parallel those in human scleroderma.

Materials and methods

Animals

C57BL/6-TSK/+ [Tsk-1, Tsk/+] and C57BL/6-pa/+ [control, +/+] mice were maintained for twelve months in our animal facility, and observed for skin changes. Marked thickening over the thoracic and cervical region was detectable in the Tsk-1 mice compared to controls.

Histological evaluation

Skin biopsies (3 mm) were obtained from the upper thoracic/lower cervical regions of 5 tight skin mice (Tsk-1) and 2 normal mice at 10 months of age. Specimens of skin from both the tight skin mice and controls were fixed in 10% neutral buffered formalin, washed

in tap water for one hour and stored in 70% ethanol. The specimens were processed manually at room temperature. The specimens were dipped in and dehydrated with increasing concentrations of ethanol (80% ethanol for 4 hours, 95% ethanol for 5 hours, 100% ethanol for 7.5 hours), then cleared with xylene (4 hours) and embedded in paraffin (paraffin infiltration for 4.5 hours, paraffin vacuum oven at 56ºF). The specimens were then sectioned at 5 μm, deparaffinized and stained with either Masson's trichrome stain (7) for evaluation of collagen, or Gomori's aldehyde fuchsin stain (8) for evaluation of elastic fibers.

Computer-assisted image analysis

For the computer-assisted image analysis of the relative proportions of collagenous and elastic tissue, sections stained for the respective components were examined with an Olympus microscope attached to a solid state camera. Selected areas were scanned with BioScan Optimas software (Media Cybernetics Inc., Silver Spring, MD, USA), utilizing a digitizer to convert the light signal to a computer-adapted format, and a personal computer was used for data analysis. Using the aforementioned apparatus, the picture analysis programs allowed the measurement of the amount of differentially stained tissue as a fraction of the total tissue in the measured field. In this portion of the study, the differentially stained collagenous and elastic tissues were made recognizable by the concept of thresholding, in which stained and unstained objects are distinguished from each other by their relative light absorption; positively stained objects will absorb a variable amount of light but at least as high as the threshold value while the unstained (negative) objects will absorb a variable amount of light but always below the threshold value. Computer generated values representing the relative percentages of collagenous and elastic tissue in the dermis of the Tsk-1 and control mice were derived and expressed in bar graph form. We analyzed five fields per section and three sections per sample.

Statistical analysis

Data were expressed as the mean ± SEM of the animals tested. The statistical significance of differences between the 2 groups was determined by 2 sample t-tests.

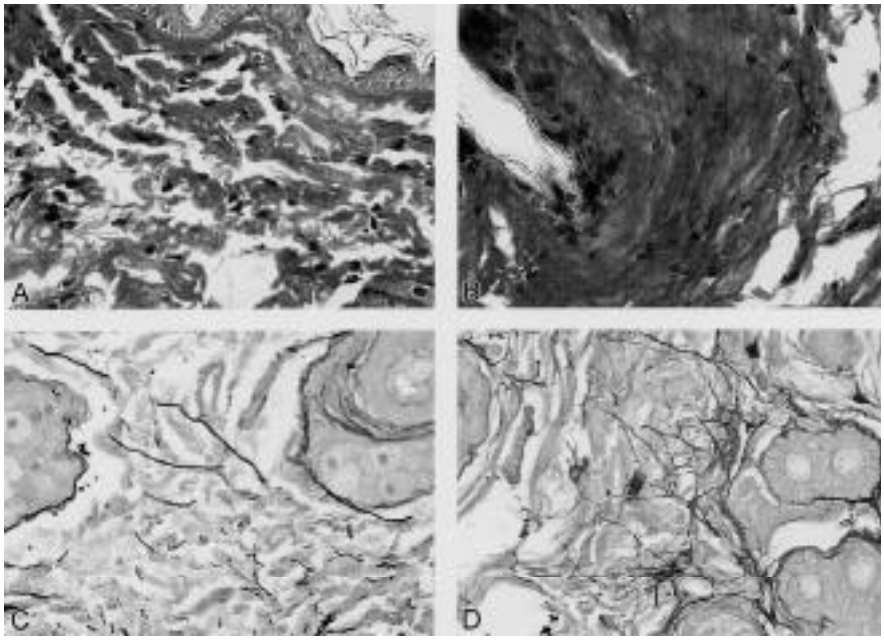

Fig. 1. Representative photomicrographs of histological findings in the dermis of a Tsk-1 mouse and a normal mouse (magnification: 4000x): Masson treated section (arrow indicates collagen fibers) showing normal mouse tissue (**A**) and Tsk-1 mouse tissue (**B**). Gomori aldehyde fuchsin treated sections (black arrow indicates elastic fibers and white arrow indicates non-elastic tissue) showing normal control tissue (**C**), and Tsk-1 mouse tissue (**D**).

Results

A representative photomicrograph of skin stained with Masson's trichrome comparing a section of Tsk-1 dermis with that of dermis from a control mouse is shown in Figures 1A and 1B. Increased collagen content may be clearly seen in sections from the Tsk-1 mouse compared to the control.

Figures 1C and 1D are photomicrographs of Gomori's aldehyde fuchsin stained sections comparing Tsk-1 dermis with control tissue. Elastic fibers form a fine, wavy meshwork within the rest of the lighter staining connective tissue. Clumps of elastic fibers are seen scattered throughout the dermis and concentrated in the lower dermis in the Tsk-1 section compared to that derived from the control animal.

In order to quantify this difference, computer assisted image analysis was used to study these samples. Comparisons of Tsk-1 and normal mice revealed substantial differences for both collagen and elastin staining. Figure 2 shows an increase in the levels of dermal collagen staining in the Tsk-1 sections (82.9 ± 9.1%) compared with the levels in normal control mice (73.7 ± 1.5%) [$p < 0.05$]. The results for collagen staining confirm previous reports (2,5). The new finding was that dermal elastin staining was also significantly increased in the Tsk-1 sections (19.6 ± 1.1%) compared with the level in normal sections (7.9 ± 0.5 %) [$p < 0.001$].

Discussion

Our studies demonstrated for the first time that there is significant increase in the elastin content of the dermis in the Tsk-1 mice compared to that in the normal controls. These observations correlate with the findings in the skin of scleroderma patients who have also been reported to have increased elastic fibers (4). As expected, collagen content as measured by trichrome staining is also increased (2, 5), reflecting changes seen in human scleroderma.

Scleroderma is characterized by an increased deposition of multiple connective tissue components in the dermis, most notably collagen. There is also an increased synthesis of elastin, which is organized in a higher number

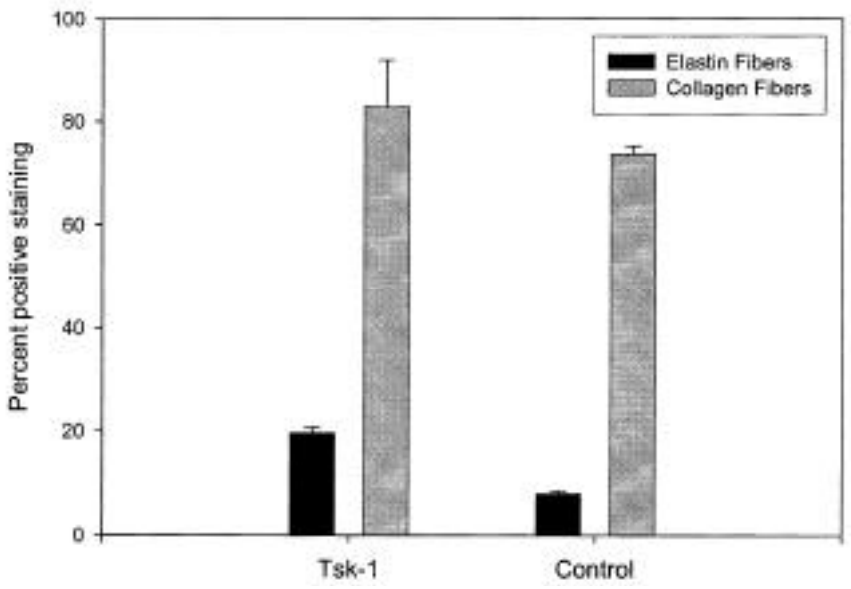

Fig. 2. Increased collagen and elastin in Tsk-1 skin. Computer-assisted image analysis quantifies the relative proportions of collagen and elastin in dermis comparing Tsk-1 mice with control mice. The data are based on the mean percentage of positive staining in 5 Tsk-1 and in 2 control mice (five fields per section x three sections per skin biopsy sample). Values are the mean ± SEM.

of fibers compared to normal skin (4, 9). This protein is extremely important for the elasticity and functional integrity of several organs.

The mechanism and pathogenesis of increased elastin deposition in scleroderma skin is still unclear. Transforming growth factor-β (TGF-β) is a multifunctional peptide growth factor that has previously been shown to enhance the synthesis of collagen, fibronectin, hyaluronate and other matrix components in various cultures of cell populations (10). It is believed that similar to very few other compounds, TGFβ is also a potent inducer of elastin synthesis in human dermal fibroblasts acting at the post-transcriptional level (4, 11-13).

One intriguing question however emerges. It is now known that in the Tsk-1 mouse, the defect lies in the mutated fibrillin gene and hence in the fibrillin-1 molecule. How can this explain the abnormalities in collagen and elastin deposition ? The studies by Saito and co-workers (14) can possibly explain this phenomenon. They demonstrated that TGF-β, which plays a crucial role in skin fibrosis, binds to both wild type and mutated fibrillin-1. The quantity of bound TGF-β is higher in mutated than in wild type fibrillin-1 and seems to be related to the number of TGF-β binding motifs. Hence it is possible that the mutated fibrillin traps an excess of TGF-β, thereby enhancing the effect of TGF-β on the fibroblasts to produce more collagen and elastin.

Similar to human scleroderma, the fibrotic skin disease in the Tsk-1 mouse is characterized by an increased number of dermal fibroblasts containing high levels of procollagen mRNA. The work of Pablos *et al.* (15) suggested that the fibrotic phenotype of the Tsk-1 mouse results neither from increased fibroblast proliferation, nor from defective apoptosis, but possibly from transcriptional activation of extracellular matrix genes.

In the Tsk-1 mouse, there are notable differences in the amount of elastic fibers in specific tissues. The decrease of elastic fibers in the lung leads to the development of emphysema (6) as opposed to pulmonary fibrosis that is seen in human scleroderma. In contrast, our experiments show that there is an increase of elastic fibers in the skin. The reasons for this discrepancy are not clear. However the dermal sclerosis is a striking finding and closely mimics the human disease.

The increased elastic fiber content in the dermis of the Tsk-1 mouse, as found in our experiments, is further evidence that the Tsk-1 mouse displays dermal connective tissue abnormalities resembling those present in human scleroderma and therefore it may be a valuable model for the study of this disease.

Acknowledgements

The authors would like to thank Ms. Lois Mayton (Orthopedic Surgery, Wayne State University) and Mr. Ruan Hangming (Department of Pathology, Wayne State University) for excellent technical assistance. The mice were purchased from Jackson Laboratories, Bar Harbor, Maine.

References

1. BOCCHIERI MH, JIMENEZ SA: Animal models of fibrosis. *Rheum Dis Clin North Am* 1990; 16: 153-67.
2. JIMENEZ SA, WILLIAMS CJ, MYERS JC, BASHEY RI: Increased collagen biosynthesis and increased expression of type I and type III procollagen genes in tight skin (TSK) mouse fibroblasts. *J Biol Chem* 1986; 261: 657-62.
3. SIRACUSA LD, MCGRATH R, MA Q *et al*.: A tandem duplication within the fibrillin 1 gene is associated with the mouse tight skin mutation. *Genome Res* 1996; 6: 300-13.
4. QUAGLINO D JR, BERGAMINI G, BORALDI F, MANZINI E, DAVIDSON JM, PASQUALI RI: Connective tissue in skin biopsies from patients suffering systemic sclerosis. *J Submicrosc Cytol Pathol* 1996; 28: 287-96.
5. JIMENEZ SA, MILLAN A, BASHEYRI: Scleroderma-like alterations in collagen metabolism occurring in the TSK (tight skin) mouse. *Arthritis Rheum* 1984; 27: 180-5.
6. O'DONNELL MD, O'CONNOR CM, FITZGERALD MX, LUNGARELLA G, CAVARRA E, MARTORANA PA: Ultrastructure of lung elastin and collagen in mouse models of spontaneous emphysema. *Matrix Biol* 1999; 18: 357-60.
7. MASSON PJ: Some histological methods: trichrome stainings and their preliminary technique. *J Tech Methods* 1929; 12: 75-90.
8. SHEEHAN DC, HRAPCHAK BB: Connective tissue and muscle fiber stains. *In* SHEEHAN DC and HRAPCHAK BB (Eds.): *Theory and Practice of Histotechnology*. The C.V. Mosby Company, 1980: 197-8.
9. FLEISCHMAJER R, JACOBS L, SCHWARTZ E, SAKAI LY: Extracellular microfibrils are increased in localized and systemic scleroderma skin. *Lab Invest* 1991; 64: 791-8.
10. PENTTINEN RP, KOBAYASHI S, BORNSTEIN P: Transforming growth factor beta increases mRNA for matrix proteins both in the presence and in the absence of changes in mRNA stability. *Proc Natl Acad Sci USA* 1988; 85: 1105-8.
11. QUAGLINO D, FORNIERI C, NANNEY LB, DAVIDSON JM: Extracellular matrix modifications in rat tissues of different ages. Corre-

lations between elastin and collagen type I mRNAexpression and lysyl-oxidase activity. *Matrix* 1993; 13: 481-90.

12. DAVIDSON JM, ZOIA O, LIU JM: Modulation of transforming growth factor-beta 1 stimulated elastin and collagen production and proliferation in porcine vascular smooth muscle cells and skin fibroblasts by basic fibroblast growth factor, transforming growth factor-alpha, and insulin-like growth factor-I. *J Cell Physiol* 1993; 155: 149-56.
13. MARIGO V, VOLPIN D, VITALE G, BRESSAN GM: Identification of a TGF-beta responsive element in the human elastin promoter. *Biochem Biophys Res Commun* 1994; 199: 1049-56.
14. SAITO S, NISHIMURA H, BRUMEANU TD *et al*.: Characterization of mutated protein encoded by partially duplicated fibrillin-1 gene in tight skin (TSK) mice. *Mol Immunol* 1999; 36: 169-76.
15. PABLOS JL, CARREIRA PE, SERRANO L, DELCASTILLO P, GOMEZ-REINO JJ: Apoptosis and proliferation of fibroblasts during postnatal skin development and scleroderma in the tight-skin mouse. *J Histochem Cyto - chem* 1997; 45: 711-9.